Centre for Clinical Microbiology, Division of Infection and Immunity,
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DNA Quality Assessment

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23/05/2022

ILOs

- Explain the principals of the techniques used for genomic DNA Quality assessment
  - DNA Quantification
  - DNA Purity
  - DNA integrity
- Apply relevant techniques to QC our DNA extracts
- Explain the Principals of DNA concentration calculations
- Apply this on our DNA extract to calculate the amounts needed per sample to pool the sequencing library at equimolar concentrations

Purity

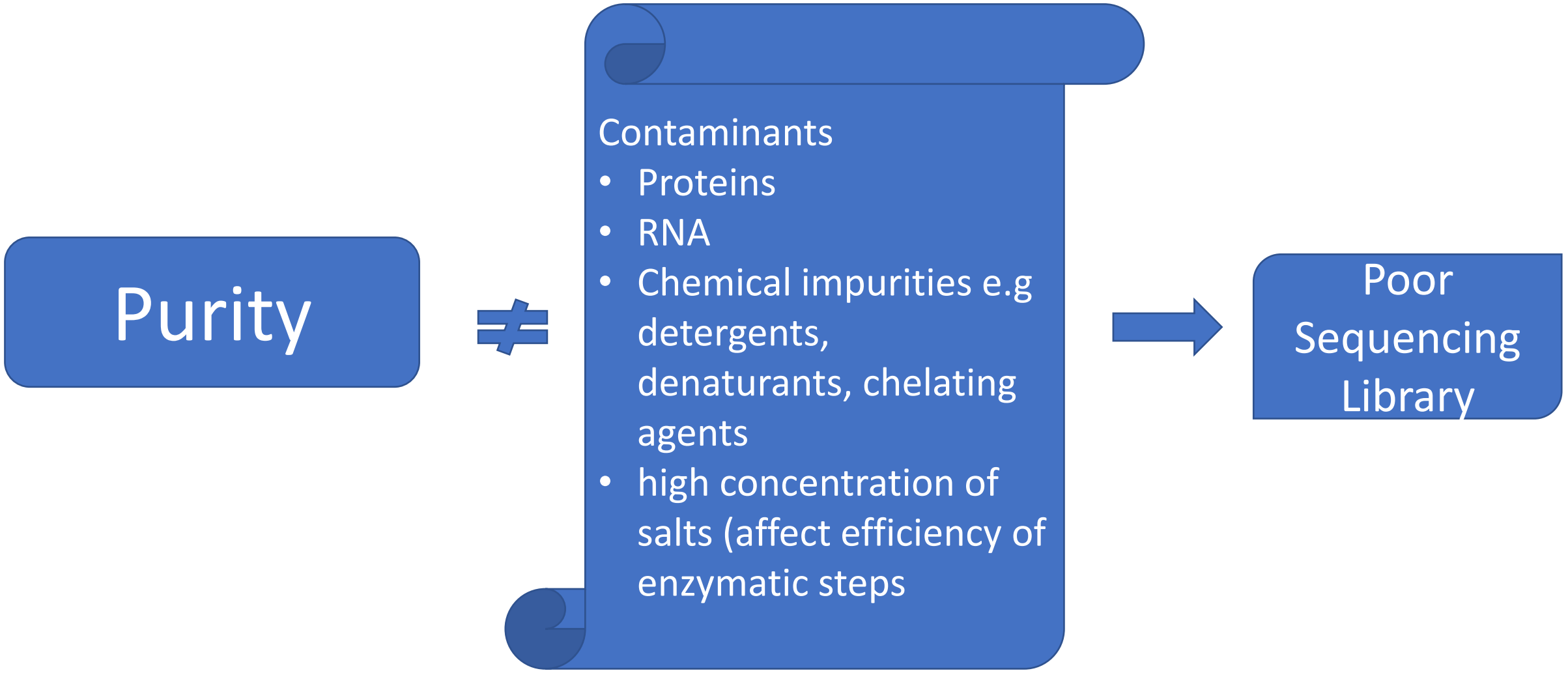
- Nanodrop™

Quantity

- Qubit ™
- Nanodrop™
- TapeStation™/Bioanalyser™

Integrity

- TapeStation™/Bioanalyser™
- Gel Electrophoresis


Purity
≠
Contaminants
• Proteins
• RNA
• Chemical impurities e.g detergents, denaturants, chelating agents
• high concentration of salts (affect efficiency of enzymatic steps
Poor Sequencing Library

Nanodrop™ Microvolume UV-Vis Spectrophotometer

- NanoDrop™ is a spectrophotometer that can be used to quantify the DNA and protein content in a 1-2 µl sample.
- RNA is a common contaminant in genomic DNA extracts
- Nanodrop cannot distinguish between DNA and RNA very well;
- Hence less accurate for quantification of DNA for library preparation purposes.
- Detection limit is 2ng/µL

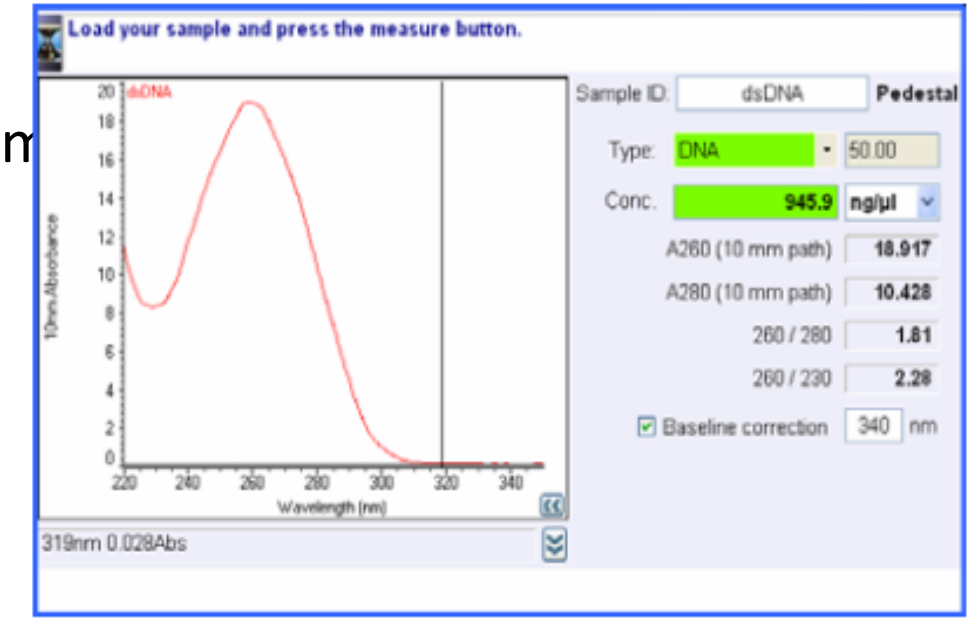
Nanodrop 2000™

Nanodrop 2000c™

Nanodrop One/One^{c}

Nanodrop™Microvolume UV-Vis Spectrophotometer

- Nucleic acids (such as DNA) absorb light at the 260 nm
- Proteins absorb light at 280 nm
- Phenolic compounds, EDTA, Carbohydrates read at 230 nm



Measure the purity of DNA extracts through measuring the

✓ A260/A280 ratio (recommended values ~1.8)
- <1.8 -> High protein content and other impurities
- (poor library preparation)
- >2 -> High RNA content

✓ A260/A230 recommended value (2.0 – 2.2)
- A260/A230 significantly <2 indicates the presence of contaminants -> purification or PCR amplification (in library prep)

Nanodrop™ Microvolume UV-Vis Spectrophotometer

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Qubit ™ Fluorometer

- High Specificity of Qubit dsDNA Assay Kits provides accurate quantification of Nucleic acids.
- Target **>53 ng/µl**
- DNA Kits
  - High sensitivity (HS) kit 0.01-100 ng/uL.
  - Broad range (BR) kit 0.2-4000 ng/uL.
- Components of the kit
  - Qubit® dsDNA HS Reagent (Component A)
  - Qubit® dsDNA HS Buffer (Component B)
  - Qubit® dsDNA HS Standard #1 (Component C)
  - Qubit® dsDNA HS Standard #2 (Component D)
  - Qubit® Assay tubes

Qubit ™ Fluorometer

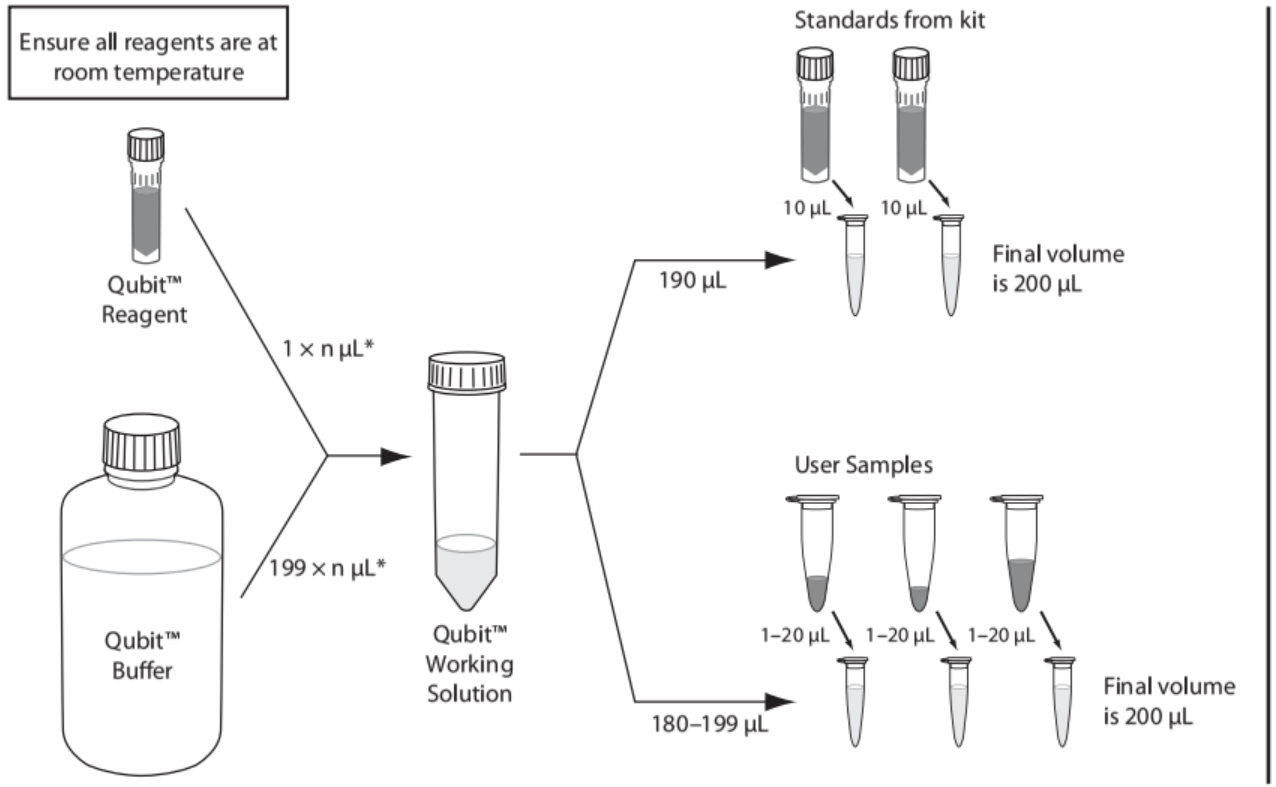
Prepare the Assay Tubes* according to the table below.

	Standard Assay Tubes	**User Sample** Assay Tubes
Volume of **Working Solution** (from step 2) to add	190 µL	180–199 µL
Volume of **Standard** (from kit) to add	10 µL	—
Volume of **User Sample** to add	—	1–20 µL
Total Volume in each Assay Tube	200 µL	200 µL

Dilution Factor 20x 1uL -> ? 200x

2uL -> ? 100x

Qubit ™ Fluorometer



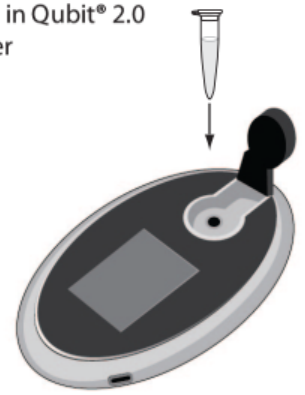
* where n = number of Standards plus number of Samples

molecular probes | invitrogen
by life technologies

Vortex all assay tubes for 2–3 seconds

Incubate at room temperature for 2 minutes (15 minutes for Qubit™ protein assay)

Read tubes in Qubit® 2.0 Fluorometer



Purity

- Nanodrop™

Quantity

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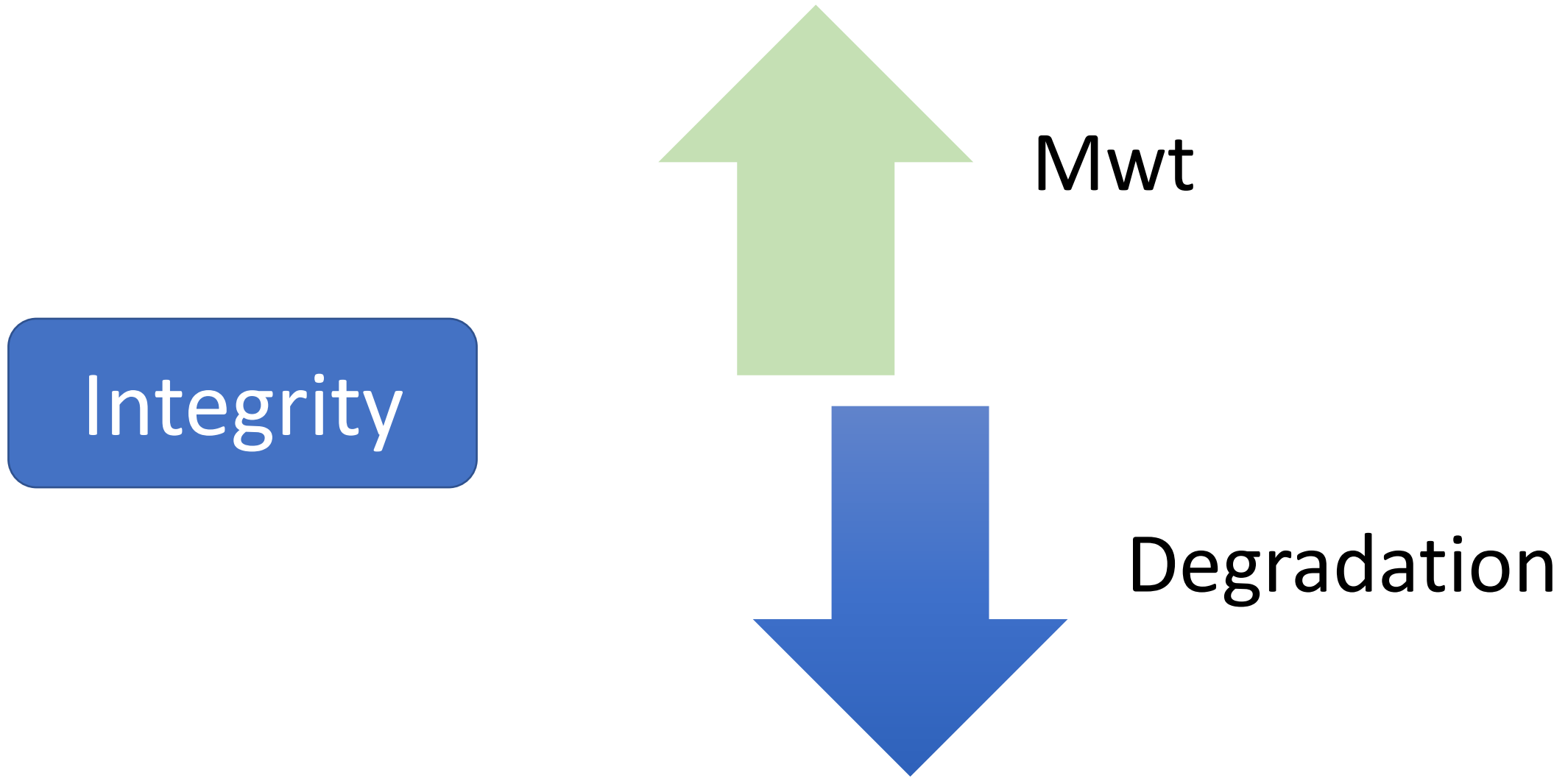
- TapeStation™/Bioanalyser™

Integrity

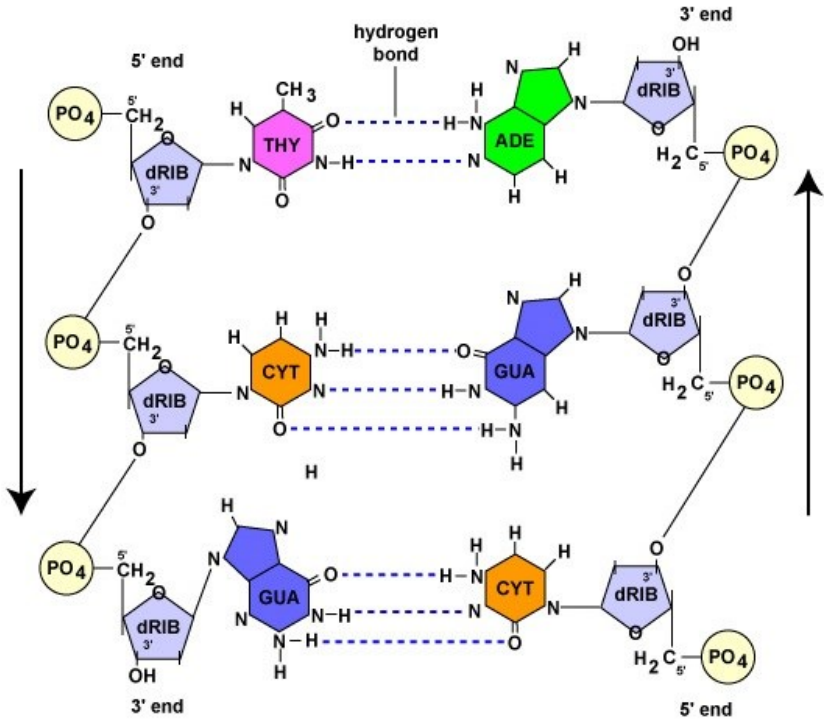
- TapeStation™/Bioanalyser™
- Gel Electrophoresis

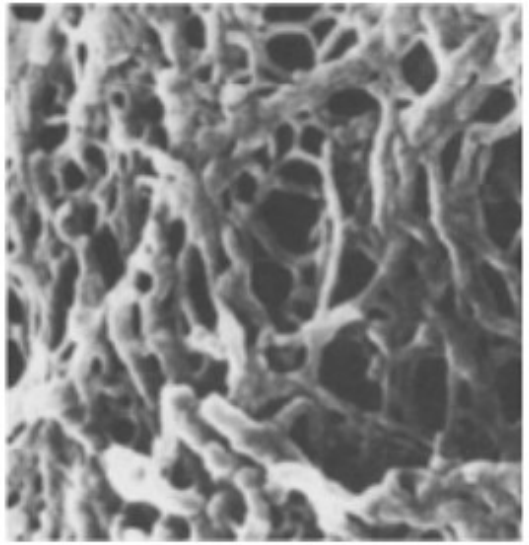

Integrity
Mwt
Degradation

Gel Electrophoresis Principal

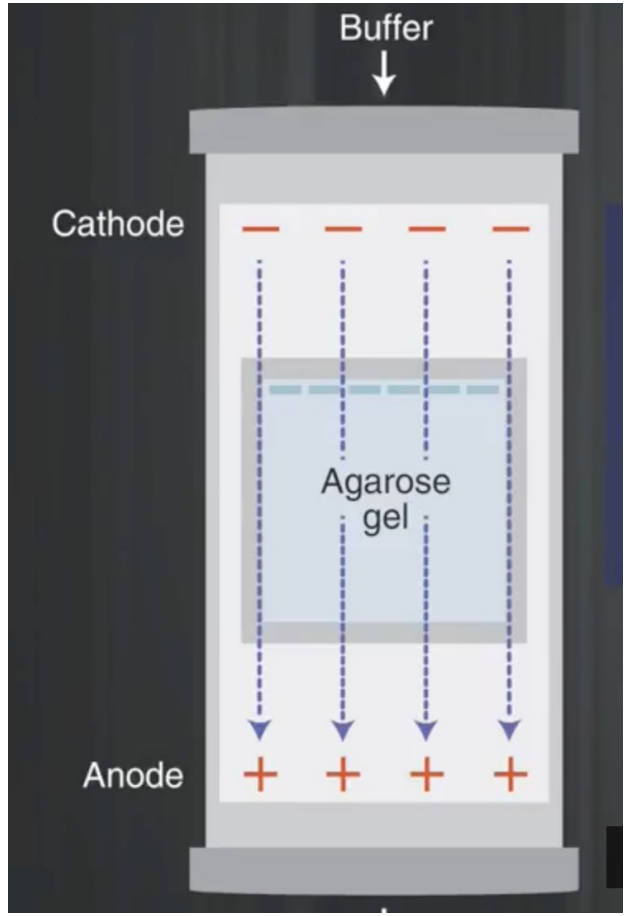
DNA is separated based on their migration rate in a gel matrix (e.g Agarose) under the influence of electric field

DNA carry charge ?

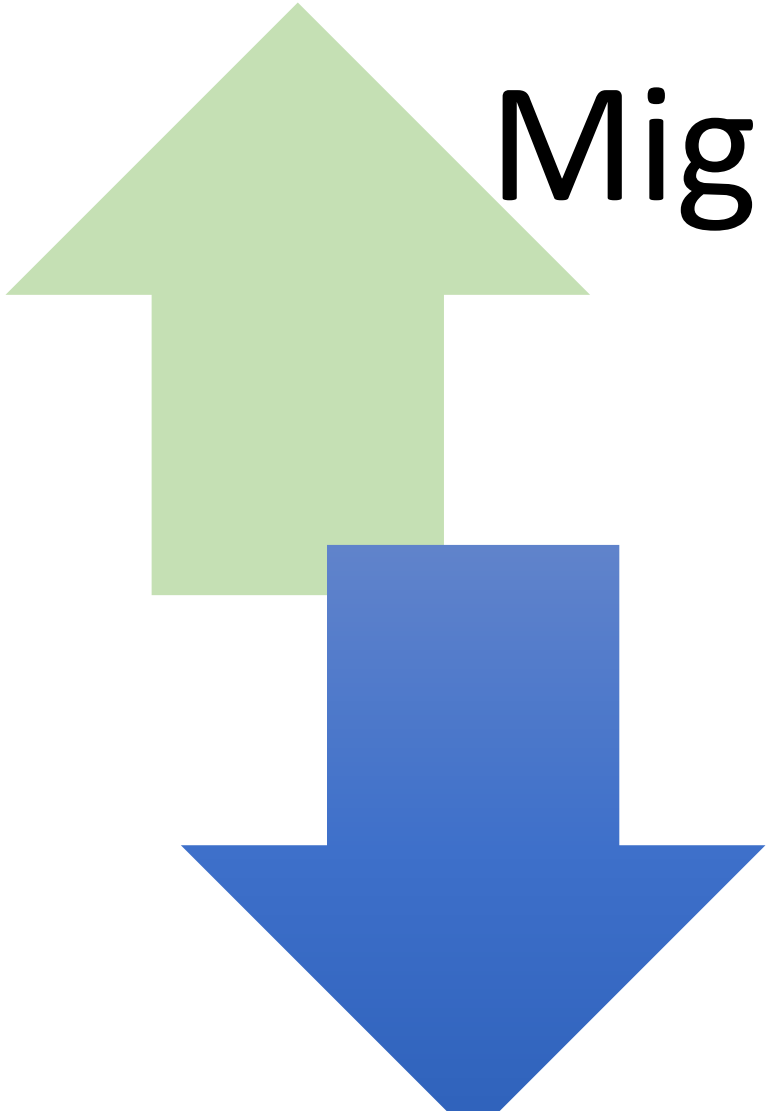




Scanning Electron Micrograph of Agarose Gel



What are Factors?



Migration Rate

- Size of the DNA fragment
- Voltage of Electric field
- Porosity of the gel matrix (Resistance)

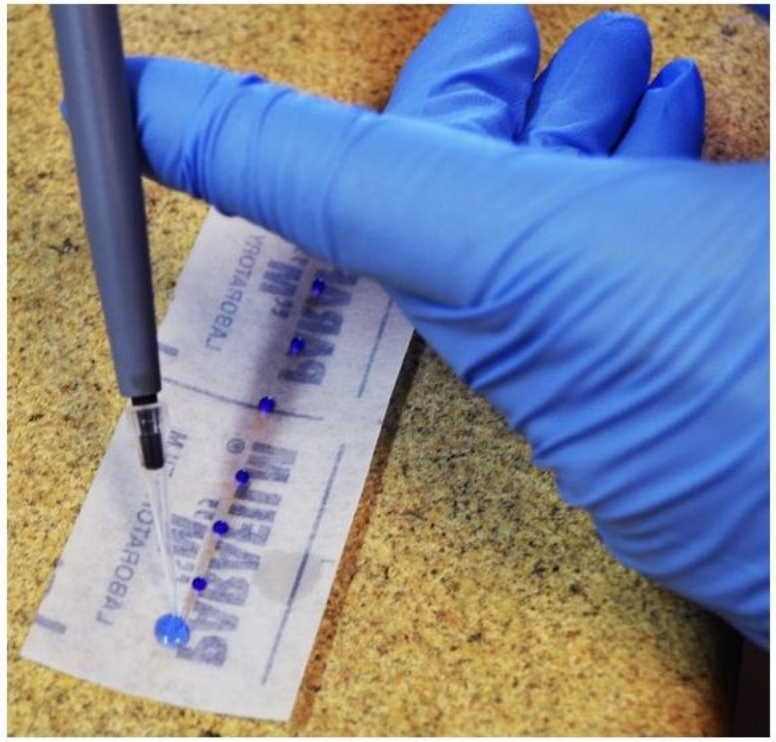
Resolution

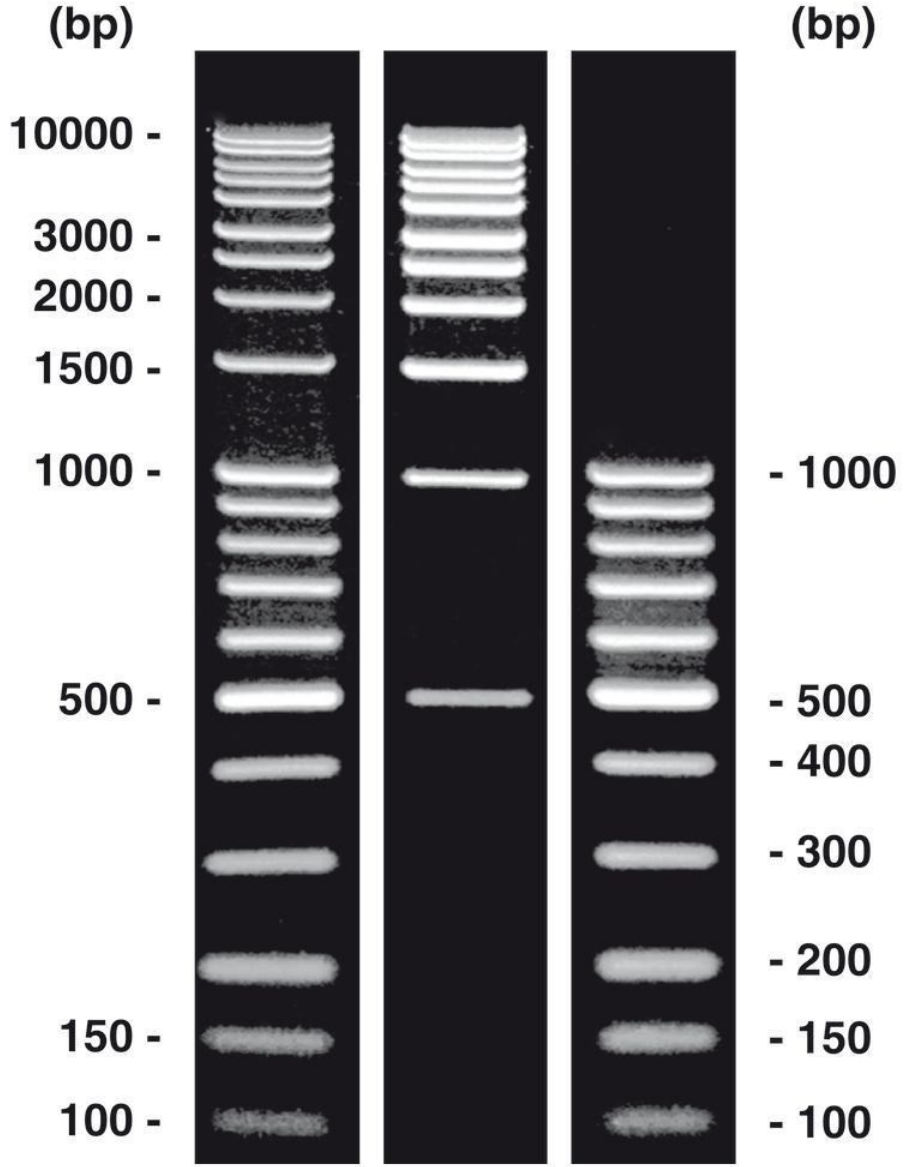
DNA Ladder + Tracking Dye

Loading Dye 6x Dilution 1:5

Tris/Borate/EDTA buffer (TBE buffer) Prepare

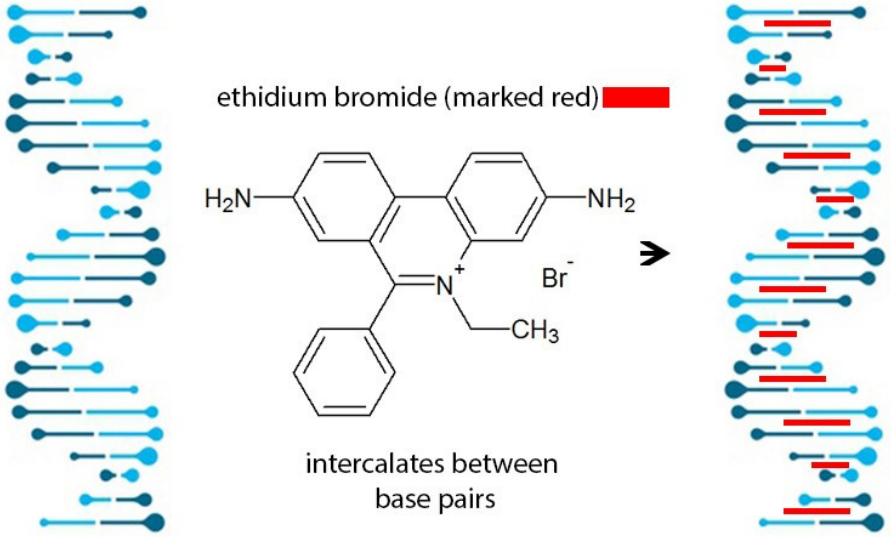
1x or 0.5x TBE buffer



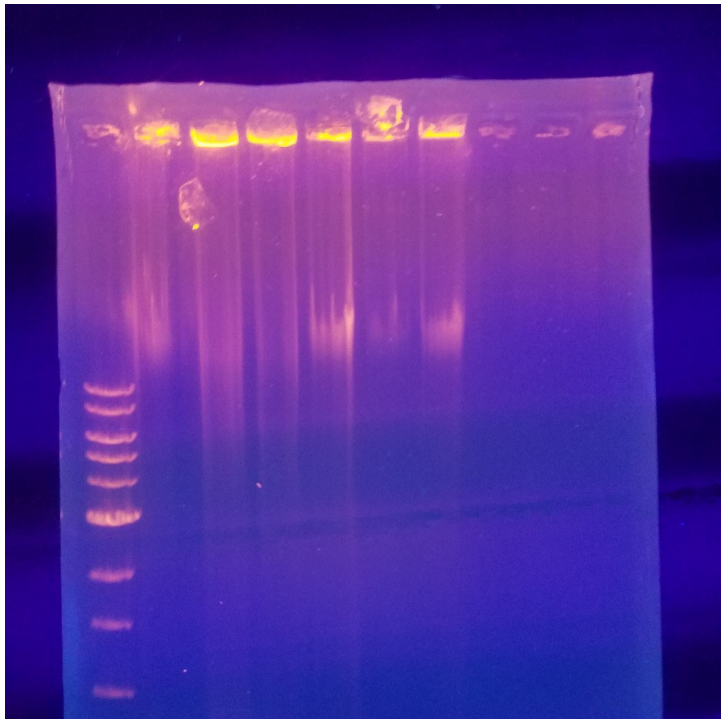


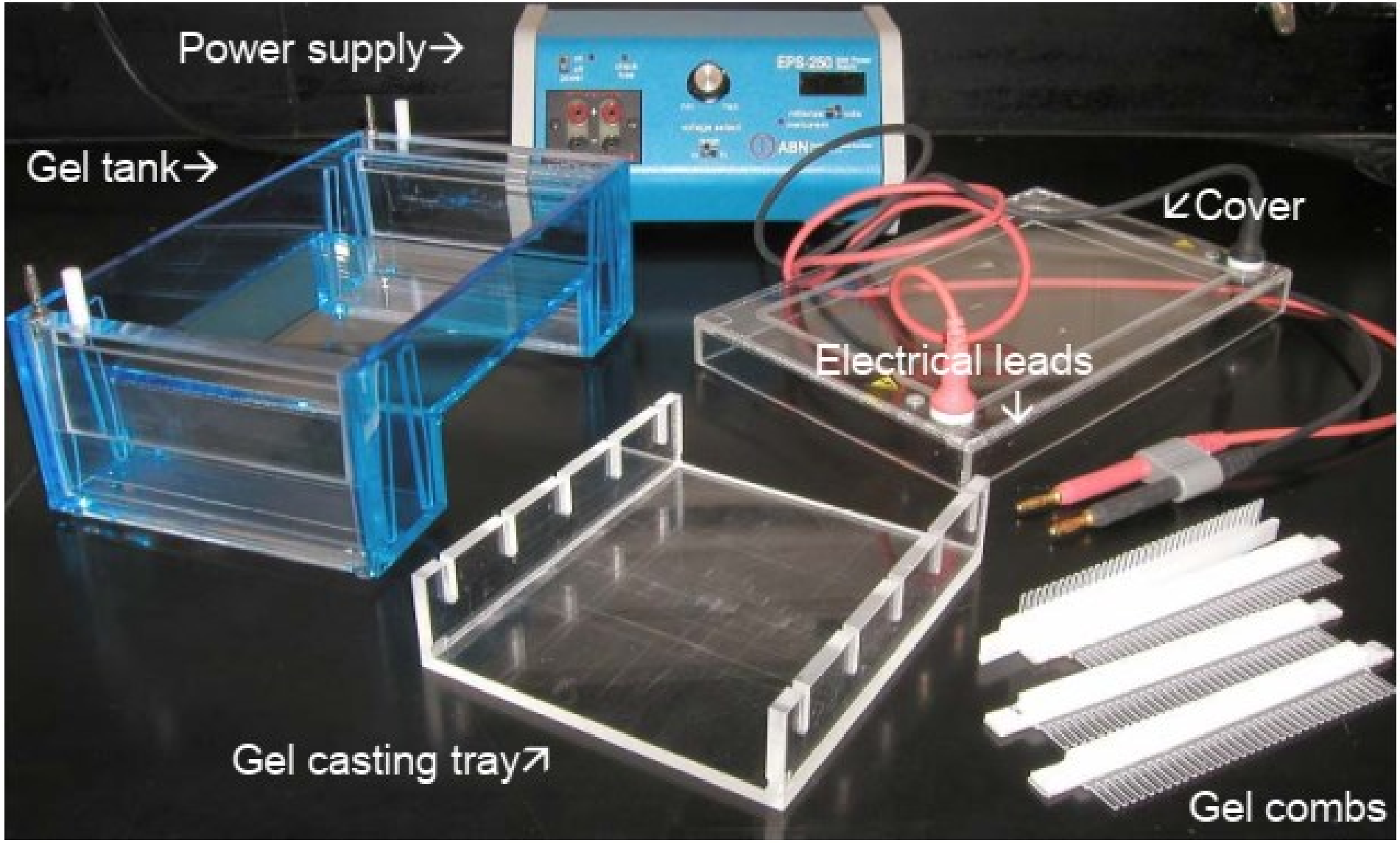
Agarose (0.7%) (? -> 100mL) (?->50 mL) TBE buffer

GelRed® (Biotium) or Ethidium Bromide



UV Lamp

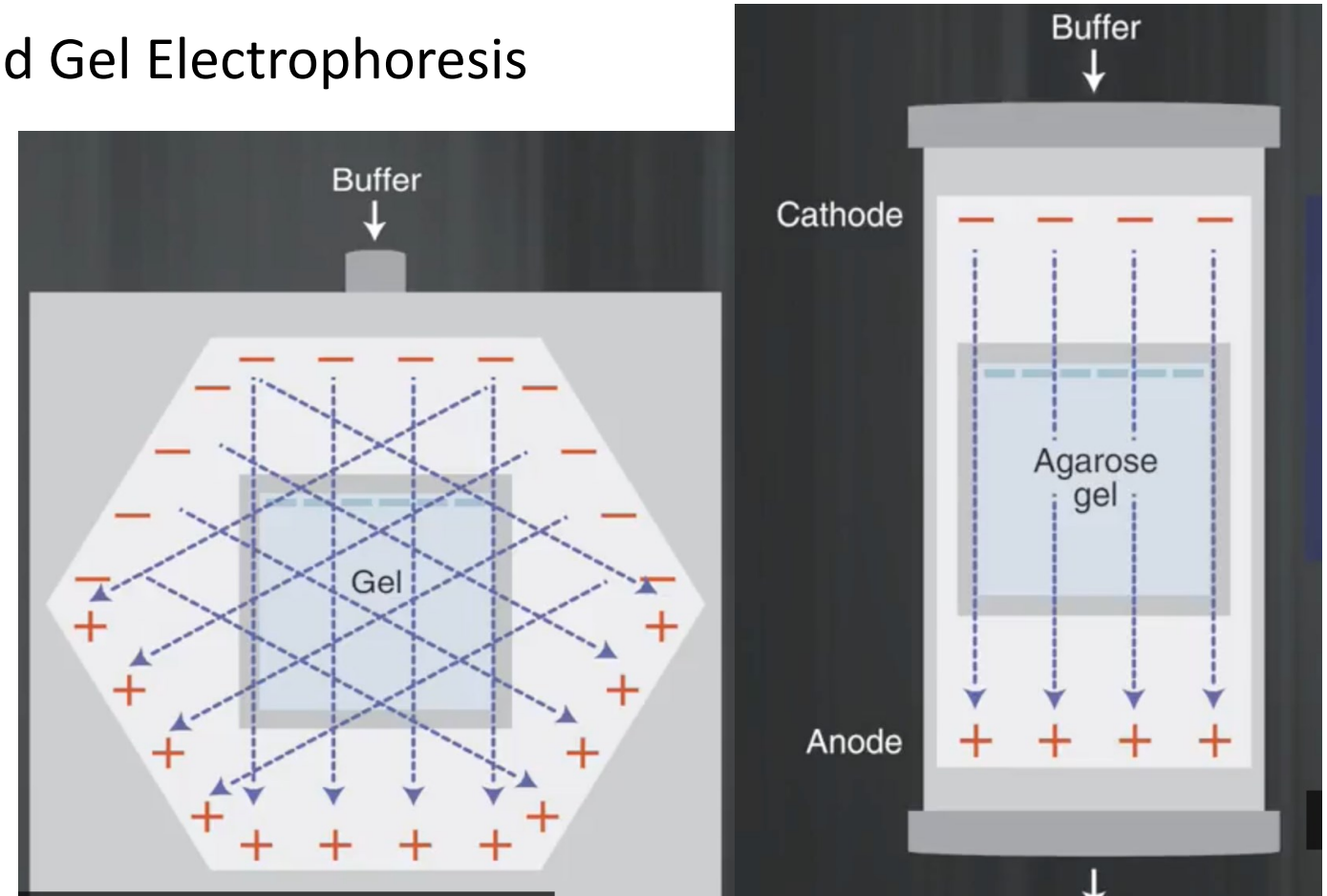



Power supply→
Gel tank→
↙Cover
Electrical leads
↓
Gel casting tray↗
Gel combs

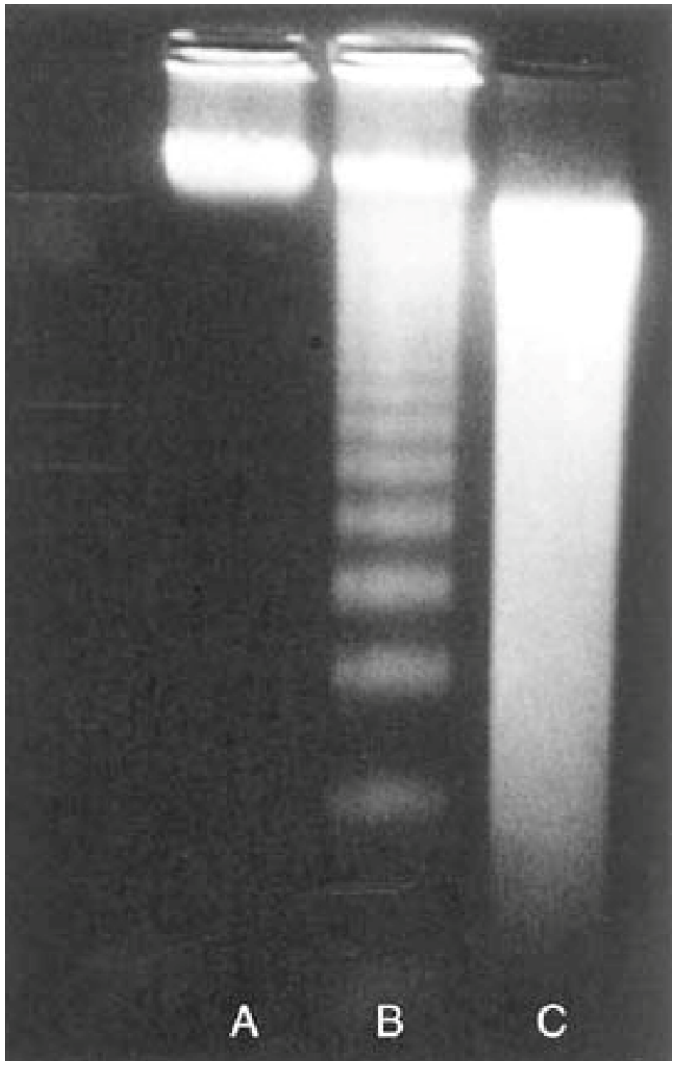
Gel Electrophoresis

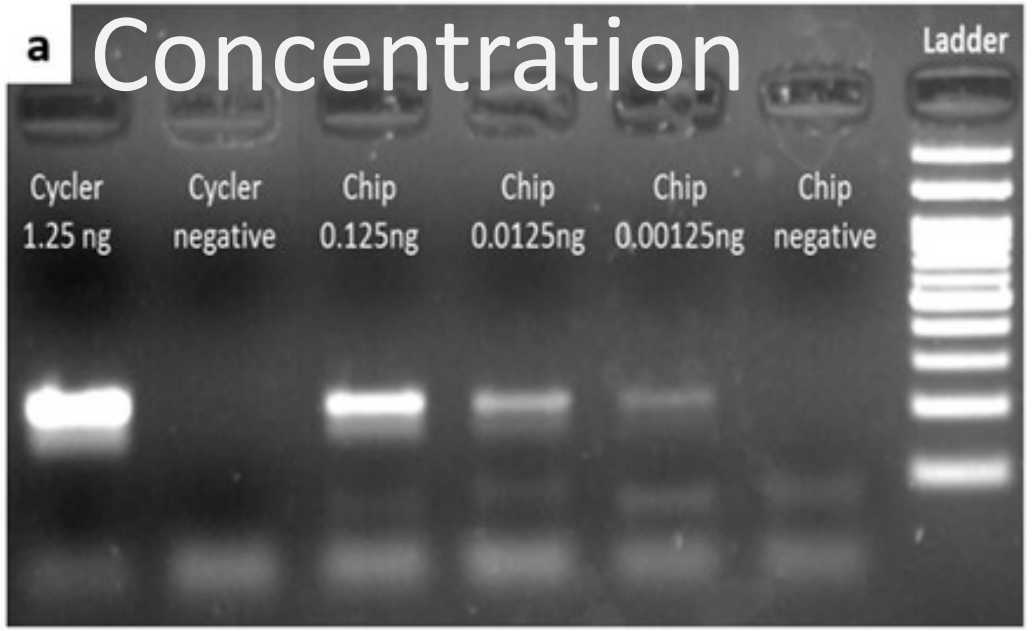
- Conventional agarose gel electrophoresis employs a static field and can resolve DNA fragments up to 50 kb
- For Resolution of larger Fragments: Pulsed Gel Electrophoresis (PFGE) is more useful

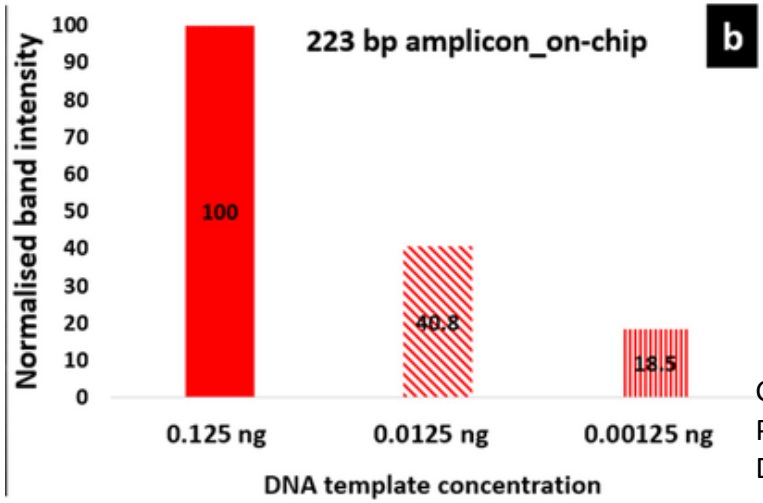
https://www.youtube.com/watch?v=k_QAXLGuQ5w



Diffuse Smearing







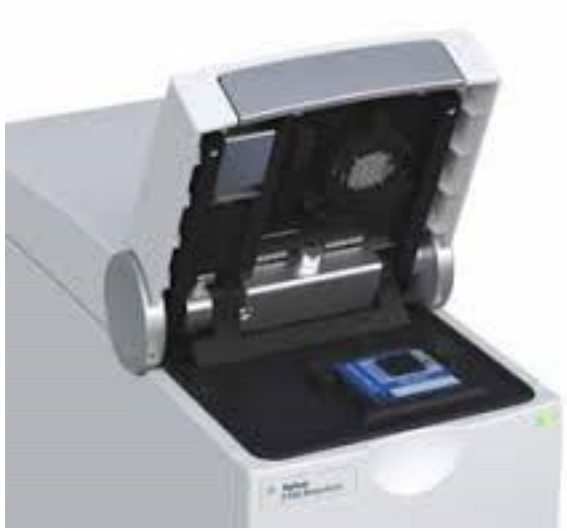
Georgia D. Kaprou et al. 2020. Towards PCB-Based Miniaturized Thermocyclers for DNA Amplification

Agilent Bioanalyzer/ TapeStation 1µL Sample

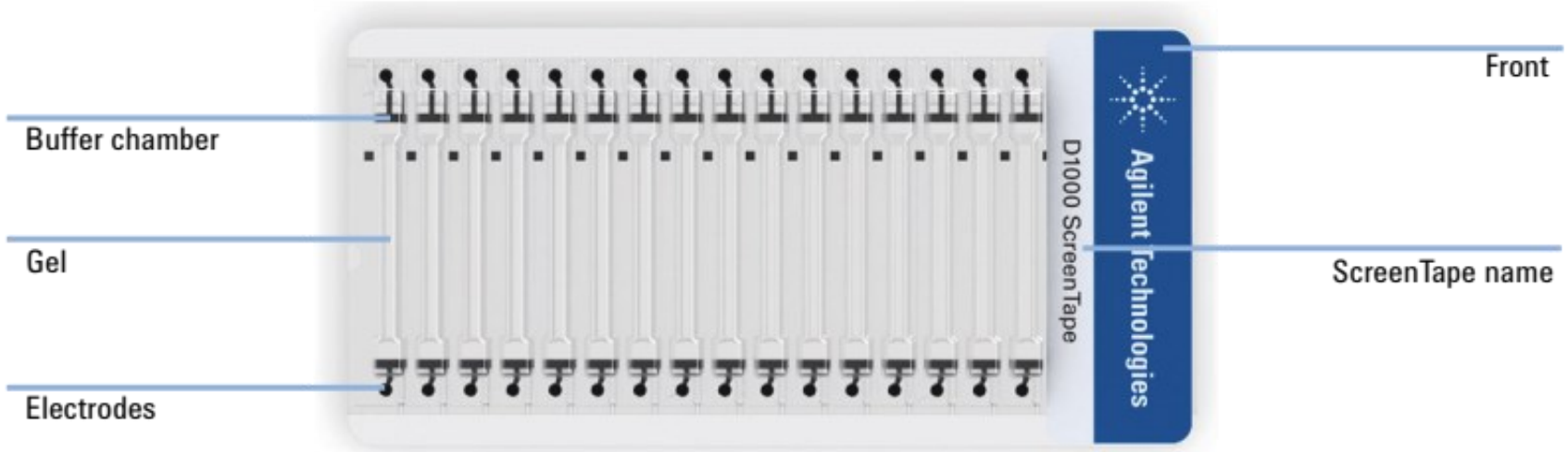
- Agilent 2100 Bioanalyzer instrument
- Chips disposable
- **Kits**
- **Agilent DNA 12000** kits are designed for the sizing and quantitation of double-stranded DNA fragments from 100 to 12000 bp.
- **Agilent DNA 7500** 100 – 7500 bp
- Can analyse 12 samples/chip, chip can not be reused.

Agilent 4200 or 2200 TapeStation
Credit card size Screen Tape 16 Samples/ Card
Can run up to 95 samples in a plate
The unused lanes in Card is reusable -> fixed cost per sample
Kits (Reagents + Screen tape):
Genomic DNA kit :Broad Range 100-60,000 bp
Agilent D1000 Kit/Agilent HS D1000 up to 1000 bp
Agilent D5000 Kit/Agilent HS D5000 up to 5000 bp

Bioanalyzer

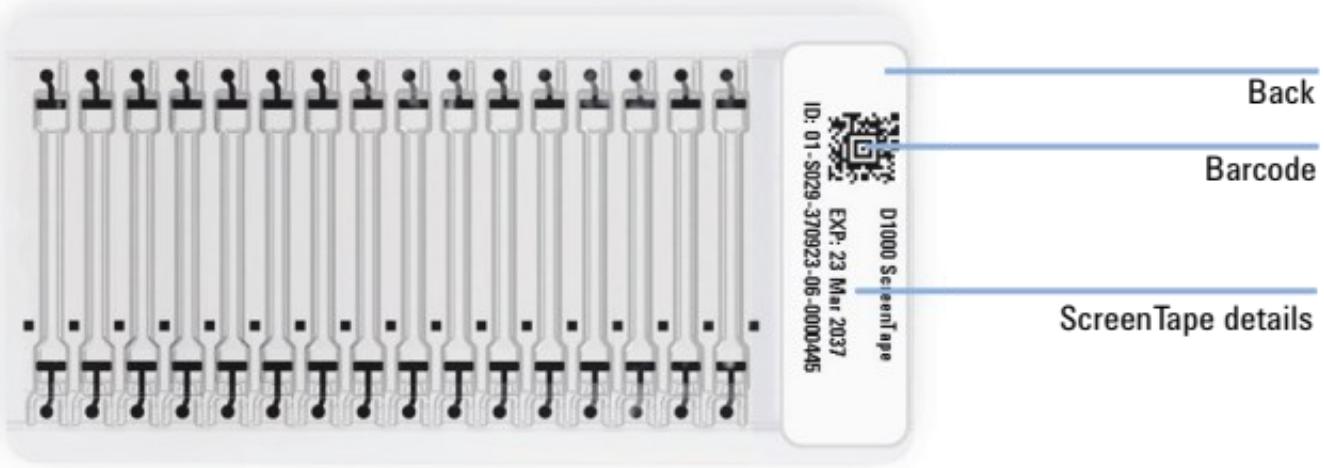


TapeStration Card Size Screen Tape



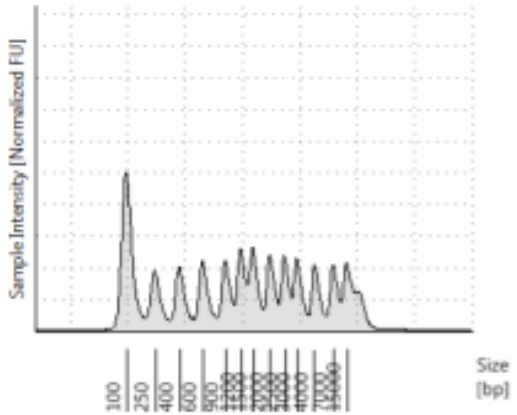
Kit Components (Genomic DNA ScreenTape Assay)

Part Number	Name	Color	Amount
5067-5365	Genomic DNA ScreenTape		7 ScreenTape devices
5067-5366	Genomic DNA Reagents		2 vials
	• Genomic DNA Ladder		25 µL
	• Genomic DNA Sample Buffer		1350 µL

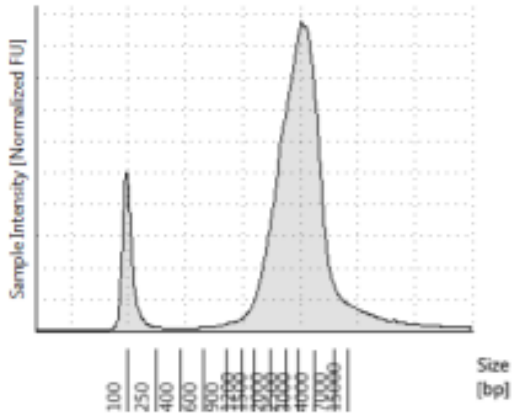


TapeStation Results

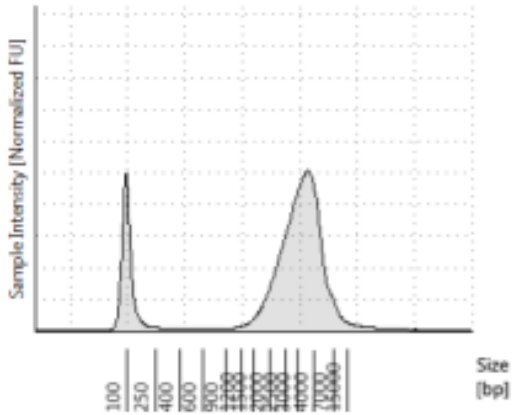
A1: Ladder



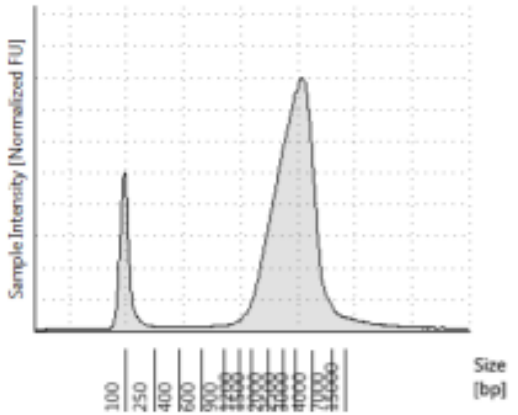
B1: 2

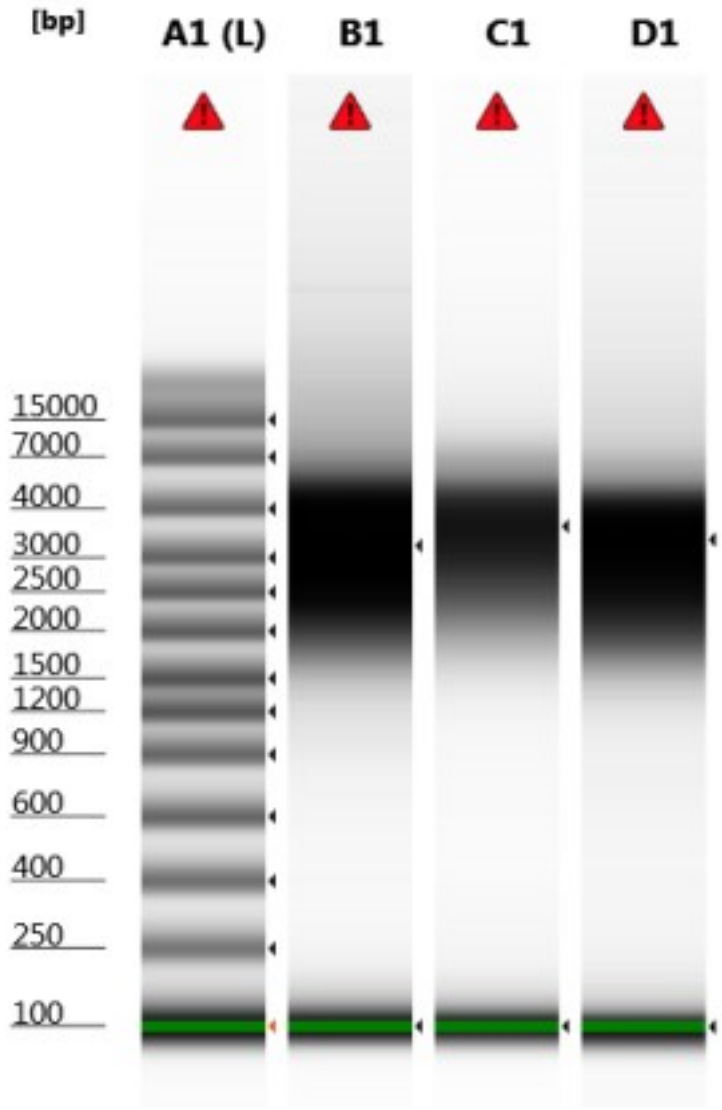


C1: A



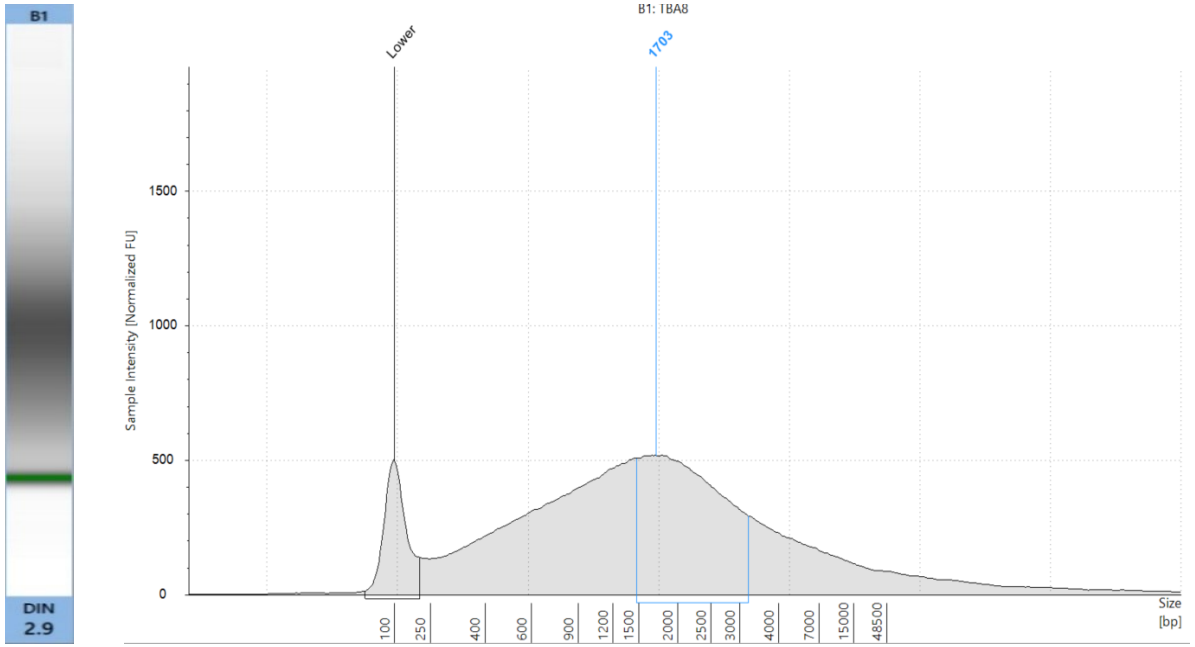
D1: C



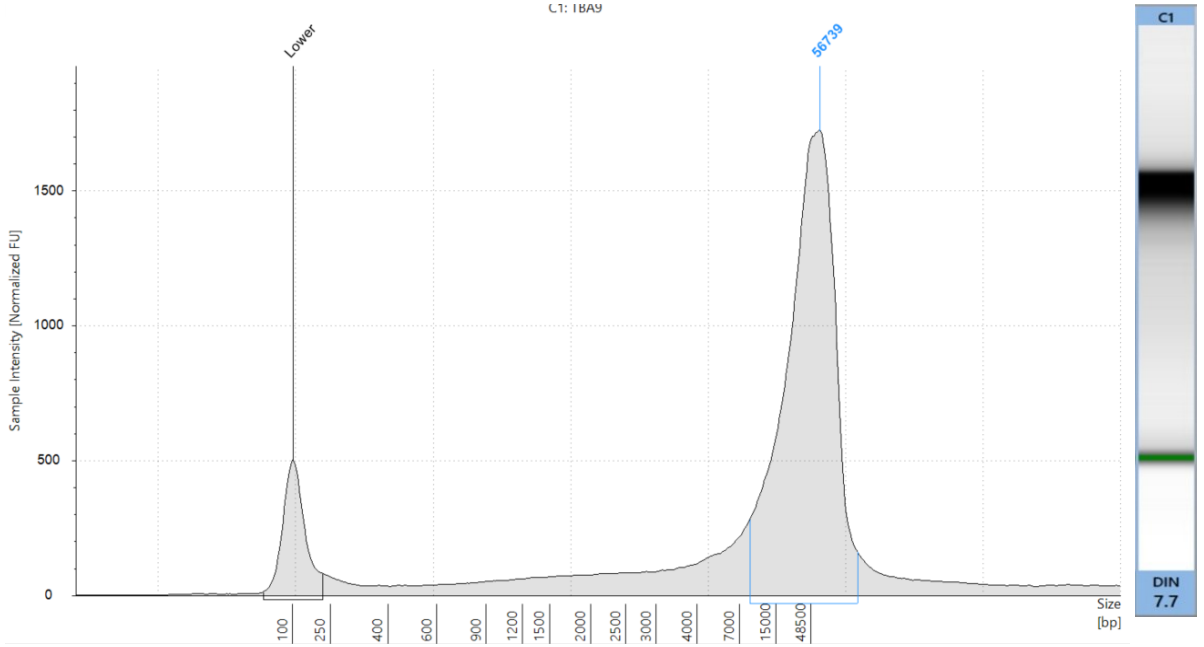


TapeStation™ Results, Which is Better?

A



B



Purity

- Nanodrop™

Quantity

- Qubit™
- Nanodrop™
- TapeStation/Bioanalyser

Integrity

- TapeStation/Bioanalyser
- Gel Electrophoresis

What to aim for?

UCL

Purity

- Nanodrop™
  - A260/A280 1.8 - <2.00
  - An Estimate of the concentration

Quantity

- Qubit™
  - Concentration >53 ng/uL

Integrity

- TapeStation/Bioanalyser
- Gel Electrophoresis
  - High Mwt genomic DNA >12 kb
  - No Degradation (Smearing, High sharp peak)

$$\text{Concentration} = \frac{weight}{Volume}$$

moles

$$\text{Molarity} = \frac{weight\ (g)}{Mwt} \times \frac{1}{volume\ (Lt)}$$

10^{-3}	=	m	milli
10^{-6}	=	μ (mu)	micro
10^{-9}	=	n	nano
10^{-12}	=	p	pico
10^{-15}	=	f	femto

Mwt of DNA Fragment =
size of a fragment (TapeStation) x average Mwt of bp (660 Da)

A mole of anything has Avogadro's number **6.022×10^{23}** of this thing

Rule: Pooling the Sequencing library at equal equimolar ratio.

What doe this practically mean?

Any Questions?

UCL